

AMENDMENTS TO THE SPECIFICATION:

Applicants request entry of the following amendments into the specification.

Please replace paragraph [00318] beginning at p. 112, with "Female Lewis rats . . ." with the following paragraph:

[00318] Female Lewis rats, 6-8 weeks of age, were purchased from ~~Charles River~~ CHARLES RIVER (Calco, Italy). EAE was induced in rats by injecting 50 µg of guinea pig MBP (~~Sigma SIGMA~~, St. Louis, MO) in water emulsified in equal volumes of complete Freund's adjuvant (CFA, ~~Sigma SIGMA~~) additioned with 7 mg/ml of heat-killed *M. tuberculosis* H37Ra (~~Difco DIFCO~~, Detroit, MI) in a final volume of 100 µ under light ether anesthesia into both hind footpads. Rats were examined in a blinded fashion for signs of EAE and scored as follows: 0, no disease; 1, flaccid tail; 2, ataxia; 3, complete hind limb paralysis with urinary incontinence. Starting from day 3 after immunization, rats were given r-Hu-EPO (EPOetin alfa, ~~Procrit, Ortho Biotech, PROCRIT, ORTHO BIOTECH~~ Raritan, NJ) intraperitoneally (i.p.) once a day at the indicated doses, in PBS. Since the clinical-grade EPO contained human serum albumin, control animals were always given PBS containing an identical amount of human serum albumin. Daily administration of 5,000 U/kg-bw of EPO increased the hematocrit by 30% (data not shown). When indicated, rats were injected s.c. once a day from day 3 until day 18 with 1.3 mg/kg-bw dexamethasone (DEX) phosphate disodium salt (~~Sigma SIGMA~~) corresponding to 1 mg/kg-bw of DEX, dissolved in PBS. When indicated, TNF and IL-6 were quantified in brain and spinal cord homogenates as previously described [Agnello, 2000 #10].

Please replace paragraph [00322] beginning at p. 114, with "Primary cultures of . . ." with the following paragraph:

[00322] Primary cultures of glial cells were prepared from new born Sprague-Dawley rats 1-2 days old. Cerebral hemispheres were freed from the meninges and mechanically disrupted. Cells were dispersed in a solution of trypsin 2.5% and DNAase 1%, filtered through a 100 µm nylon mesh and plated (140,000 cells per 35 mm dish) in Eagle's minimum essential medium supplemented with 10% fetal calf serum, 0.6% glucose, streptomycin (0.1 mg/ml) and penicillin (100 UI/ml). Glial cultures were fed twice a week and grown at 37° C in a humidified incubator with 5% CO₂. All experiments were performed on 2-3 week-old glial cell cultures with 97% astrocytes and 3% microglia, as assessed by immunochemistry [[o]] of GFAP and *Griffonia*

simplicifolia isolectin B₄. Neuronal cultures were established from the hippocampus of 18-day rat fetuses. Brains were removed and freed from meninges and the hippocampus was isolated. Cells were dispersed by incubation for 15-20 min at 37° C in a 2.5% trypsin solution followed by tituration. The cell suspension was diluted in the medium used for glial cells and plated onto polyornithine-coated coverslips at a density of 160,000 cells per coverslip. The day after plating, coverslips were transferred to dishes containing a glial monolayer in neuron maintenance medium (Dulbecco's DULBECCO'S modified Eagle's medium and Ham's nutrient mix F12 supplemented with 5 µg/ml insulin, 100 µg/ml transferrin, 100 µg/ml putrescine, 30 nM Na selenite, 20 nM progesterone and penicillin 100 U/ml) supplemented with cytosine arabinoside 5 µM. Coverslips were inverted so that the hippocampal neurons faced the glia monolayer. Paraffin dots adhering to the coverslips supported them above the glia, creating a narrow gap that prevented the two cell types from contacting each other but allowed the diffusion of soluble substances. These culture conditions allowed the growth of differentiated neuronal cultures with >98% homogeneity, as assessed by immunochemistry of microtubule-associated protein 2 and GFAP. Cells were then treated for 24 hours with 1 µM Trimethyl tin (TMT), in the presence or absence of rhEPO (10 U (80 ng)/ml), the supernatants used for TNF assay and cellular viability evaluated as described below. When indicated, glial cells were cultured in the presence of LPS for 24 hours, with or without rhEPO, and TNF measured in the cultured supernatants. Cell viability was measured by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Denizot, F., and Lang, R. 1986. Rapid colorimetric assay for cell growth and survival. Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods* 89:271-277. Briefly, MTT tetrazolium salt was dissolved in serum-free medium to a final concentration of 0.75 mg/ml and added to the cells at the end of the treatment for 3 h at 37° C. The medium was then removed and the formazan was extracted with 1N HCl:isopropanol (1:24). Absorbance at 560 nm was read on a microplate reader.

Please replace paragraph [00323] beginning at p. 115, with "**Figure 29** shows. . ." with the following paragraph:

[00323] **Figure 29** shows that rhEPO prevents neuronal death-induced TNF production in mixed neuron-glia cultures. Panel A: Percentage of neural cell death induced by TMT 1 µM without or with treatment with rhEPO (10 U/ml). Panel B: Release of TNF[[-__]] from

glial cells exposed to TMT 1 μ M in the presence (hatched bars) or absence (filled bars) of neurons, with or without rhEPO (10 U/ml). Similar results would be expected from the therapeutic treatment with the tissue protective cytokines of the present invention.